# Dicarbonyl-nitrosyl-complexes of rhenium (Re) and technetium (Tc), a potentially new class of compounds for the direct radiolabeling of biomolecules

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## *Short Communication*

# Dicarbonyl-Nitrosyl-Complexes of Rhenium (Re) and Technetium (Tc), A Potentially New Class of Compounds for the Direct Radiolabeling of Biomolecules

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*Re- and Tc-complexes of the oxidation state (+I) offer a useful synthetic pool for the labeling of biomolecules due to their co-ordination properties and stability, which are superior to compounds of the oxidation state (+V). Based on the results for Tc-tricarbonyl complexes it was the topic of this work to develop an access to similar but higher charged compounds, which could be performed by replacing a neutral [CO]-group by a $[NO]^+$-group. The resulting Re(I)- and Tc(I)-dicarbonyl-nitrosyl complexes, such as $[N(CH_2CH_3)_4][ReX_3(CO)_2(NO)]$, show a tendency for co-ordination at carboxylic and amine groups of biomolecules (X = Br, Cl). This was shown with picolinic acid (H-pic), a suitable model for amino acids, forming the neutral complex $[ReX(pic)(CO)_2(NO)]$. In a similar fashion conjugation of $[^{188}Re(CO)_2(NO)]^{2+}$—or $[^{99m}Tc(CO)_2(NO)]^{2+}$ —compounds to proteins or antibodies is feasible. This approach opens a way to a potentially new class of radiopharmaceuticals.*

**Key words:** Rhenium, nitrosyl complexes, carbonyl complexes, radiopharmaceuticals

## INTRODUCTION

The chemistry of rhenium and technetium has received much attention due to the importance of the isotopes Re-188 and Tc-99m for therapeutic and diagnostic purposes, respectively.[1,2] Tc-99m with a half-life of 6 hours is most attractive for diagnostic purposes, since it is readily and economically available through a $^{99}Mo/^{99m}Tc$ generator system and has an ideal photon energy. Re-188 with a half-life of 16.9 hours is also a generator-produced radioisotope obtained from an alumina-based $^{188}W/^{188}Re$ generator system currently under evaluation for a variety of therapeutic applications, including bone pain palliation and intravascular radiation therapy.[3] Complexes of the oxidation state (+V) have been

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widely used since they are easily accessible by reduction of the corresponding permetallates $[MO_4]^-$ (M = $^{188}$Re, $^{99m}$Tc), e.g., with $SnCl_2$. However complexes of the oxidation state (+I) have not yet been explored to any great extent for clinical purposes. Since the successful introduction of $[^{99m}Tc(CN\text{-}R)_6]^+$ in scintigraphy the chemistry of Re- and Tc-compounds in low oxidation states has become more popular.[4] Carbonyl compounds with a $[M(CO)_3]^+$-moiety especially, have shown remarkable potential due to their co-ordination properties, enabling them to form complexes with nitrogen containing biomolecules in high specific activity. They possess a $d^6$ low-spin electronic configuration and are kinetically very stable, since the dissociative ligand substitution pathway is quantum mechanically forbidden. Associative substitution is strongly disfavored because of the lack of metal orbitals available for additional interaction with donating or accepting ligands.[5] The goals of studies described in this paper were to demonstrate the usefulness of Re(I)- and Tc(I)-dicarbonyl-nitrosyl complexes as alternatives to tricarbonyl compounds.

## MATERIALS AND METHODS

Although such metal nitrosyl complexes are well known in organometallic chemistry, they have not been used in the clinic.[6] Their clinical use has suffered from lack of readily accessible precursors, which could only be obtained under difficult reaction conditions including high-pressure, high-temperature, organic solvents and aggressive gases like $Cl_2$ and NO.[7] Recently, a convenient synthesis was reported to overcome this limitation where $[^{99m}Tc\ (CO)_3(OH_2)_3]^+$ was prepared under mild reaction conditions (1 atm CO, $NaBH_4$, 30 min at 75°C) starting from generator eluated $[^{99m}TcO_4]^-$.[8] Tricarbonyl complexes can now serve as starting materials to generate a $[M(CO)_2\ (NO)]^{2+}$-moiety (Fig. 1). For example $[N(CH_2CH_3)_4]_2[^{99m}TcX_3(CO)_3]$ can easily be converted into $[N(CH_2CH_3)_4][^{99m}TcX_3(CO)_2\ (NO)]$ with $[NO][BF_4]$ in acetonitrile or dichloromethane (X = Br, Cl).[9,10]

With the synthesis of tricarbonyl complexes under mild conditions, the promising properties of the nitrosyl ligand can now be explored for clinical purposes. $[NO]^+$ is isoelectronic with CO, but is a better $\pi$-acceptor and thus better stabilizes $\pi$-donating ligands in the *trans*-position. Due to Pearson's concept of hard and soft acids and bases ("HSAB" principle) the metal center becomes harder and acquires a preference for reaction partners like oxo- or nitrogen-groups.[11] So, for example, antibodies or peptides which include amino acids become possible targets for direct labeling. In addition a nitrosyl ligand, as opposed to a carbonyl ligand, causes a bigger split of the HOMO-LUMO gap, thus increasing the stability of the complex (HOMO = highest occupied molecular orbital; LUMO = lowest unoccupied molecular orbital). Finally, the charge of the complex is changed by +1 creating new characteristics, for instance, for the co-ordination of further ligands, biodistribution of labeled compounds or a higher acidity in aqueous solution.

To exemplify the potential of low spin $d^6$ complexes with a $[M(CO)_2(NO)]^{2+}$-moiety as a label for biomolecules, the reaction of $[N(CH_2CH_3)_4]$ $[MX_3(CO)_2(NO)]$ with picolinic acid (H-pic) was investigated in detail (M = Re, Tc and X = Br, Cl). Picolinic acid can be considered a suitable model since it provides two coordinating sites, an aromatic amine and a carboxylic acid. In fact it co-ordinates bidentate to the metal center thus forming $[MX(pic)(CO)_2(NO)]$.[9,12] Picolinic acid deprotonates when coordinating to the metal cen-

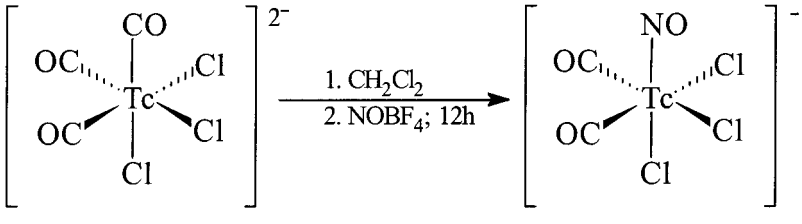


**Figure 1.** Synthesis of $[^{99m}TcCl_3(CO)_2(NO)][N(CH_2CH_3)_4]$

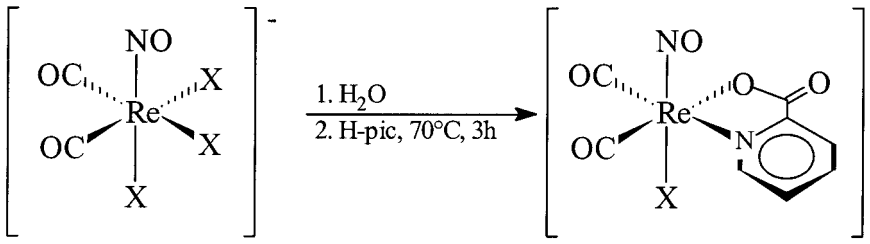


**Figure 2.** Synthesis of $[ReX(pic)(CO)_2(NO)]$ (X = Br, Cl)

ter and the neutral complex precipitates in water. The coordination of the bidentate ligand occurs *trans* to both carbonyl groups while the position *trans* to the nitrosyl group is occupied by a halide (Fig. 2). In saline solution the position *trans* to the NO-ligand in this complex will be occupied by a chloride.

The same complex could be obtained in a reaction of $[N(CH_2CH_3)_4]_2$ $[MX_3(CO)_3]$ with picolinic acid, forming the anionic compound $[N(CH_2CH_3)_4][MX(pic)(CO)_3]$, and subsequent nitrosylation with [NO] $[BF_4]$ in dichloromethane. Substitution of the third CO-group selectively occurs *trans* to the remaining halide, indicating the preference for a X-M-NO axis with a $\pi$-donating ligand *trans* to the nitrosyl group as pointed out above. Crystallization from a mixture of dichloromethane/hexane yielded yellow crystals that are stable to air and suitable for X-ray diffraction studies.[9,13] An ORTEP drawing is depicted in Figure 3.

As expected the Re-CO distance *trans* to the acid group of picolinic acid (1.824 Å) is shorter than that *trans* to the amine of the heterocycle (1.991 Å). It is interesting that the Re-CO distance is even shorter than the Re-NO distance in the complex (1.893 Å).

Additional experiments with picolinamine (pica), a ligand which can provide an aromatic and a primary amine for coordination, showed similar results, forming the cationic complex $[MX(pica)\ (CO)_2\ (NO)]^+$.[9,14] Equal to the complex with picolinic acid the position *trans* to the nitrosyl group is occupied by a halide and both nitrogen groups of the bidentate ligand coordinate *trans* to the carbonyl groups. Biologically more relevant ligand systems like histidine and methionine were also labeled and characterized, but have not yet been crystallized, thus we didn't present them in this paper. Investigations with analogous sulfur containing ligands have been presented elsewhere and show further possibilities for direct labeling.[15]

## RESULTS AND DISCUSSION

These results suggest that direct labeling of underivatized antibodies or peptides with certain $^{188}$Re- or $^{99m}$Tc-dicarbonyl-nitrosyl complexes is feasible, leading to a $[MX(Ab)(CO)_2(NO)]$ radioimmunoconjugate (Ab = antibody or peptide). The resulting complexes are expected to be stable in air and water, which is a major requirement for use. As described for Re- and Tc-tricarbonyl complexes, the small size of the $[M(CO)_2(NO)]^{2+}$-moiety affords minimum interference with the binding site of a biomolecule, thus increasing the chance it will retain biological activity.[16] In this way dicarbonyl-nitrosyl complexes offer a new synthetic pool for direct labeling of biomolecules.

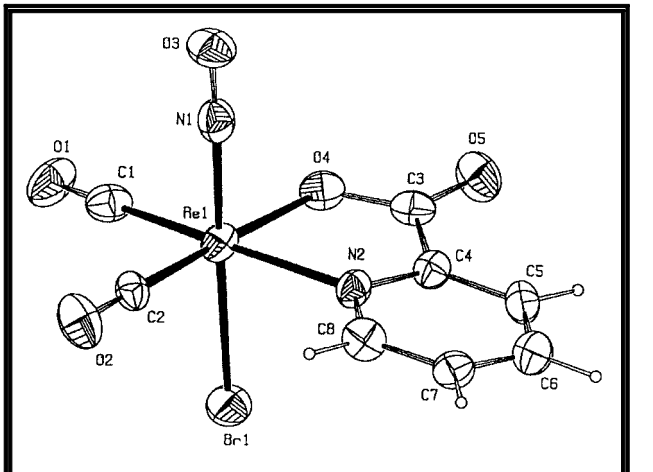


**Figure 3.** ORTEP drawing of $[ReBr(pic)(CO)_2(NO)]$

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10. Typical procedure: $[N(CH_2CH_3)_4]_2$ $[^{99}TcX_3(CO)_3]$ (153 mg, 0.275 mmol) was placed in a Schlenk tube equipped with a gas bubbler, dissolved in 15 ml dry dichloromethane and stirred for 30 min at room temperature under nitrogen. To this white suspension $[NO][BF_4]$ (33 mg, 0.28 mmol) was added. The reaction mixture became yellow and clear during 2 h. After 5 h 50–60% of the product $[N(CH_2CH_3)_4]$ $[^{99}TcX_3(CO)_2(NO)]$ could be detected by TLC (silica gel, methanol/HCl conc. (99/1 v/v), $R_f$ = 0.15), after 24 h 70–85%. Dichloromethane was removed under vacuum, the orange residue dissolved in 5 ml THF, filtered and evaporated to dryness. Yield 70–90%.
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12. Typical procedure: $[N(CH_2CH_3)_4]$ $[ReBr_3(CO)_2(NO)]$ (100 mg, 0.156 mmol) and 2-picolinic acid (20 mg, 0.16 mmol) were dissolved in 4 ml $H_2O$ and stirred for 12 h at 70°C. Parts of the neutral complex $[MBr(pic)(CO)_2(NO)]$ precipitated (14 mg, 0.0296 mmol, 19%). After another 12 h the solution was evaporated to dryness, the product extracted with THF from the residue, filtered, and evaporated to dryness again.

Overall yield of the yellow crystalline complex 70–80%.

13. Experimental data for [ReBr(pic)$(CO)_2$(NO)]: IR (KBr): $\upsilon$ [$cm^{-1}$]= 2105, 2034 (vs, CO), 1777 (vs, NO), 1685 (s, carboxylic acid); MS (EI): 474 ($M^+$), 446, 418, 388; $^1$H-NMR ($CDCl_3$): $\delta$ [ppm] = 8.80 (m, 1 H, H-6), 8.57 (m, 1 H, H-4), 8.32 (m, 1 H, H-3), 8.23 (m, 1 H, H-5); Analysis for $BrC_8H_4N_2O_5Re$: calc.: C: 20.26; H: 0.85; N: 5.91; found: C: 20.32; H: 0.87; N: 5.73; Crystal data: MW = 474.24, monoclinic, $P2_1/n$, a = 8.487(10) Å, b = 13.250(13) Å, c = 10.321(14) Å, V = 1159.4(2) $Å^3$, Z = 4, $D_{calc}$ = 2.717 g/$cm^3$, $\mu$ = 13.937 $mm^{-1}$, Siemens (Nicolet) R3m/V2000 diffractometer, Mo $K_\alpha$ radiation ($\lambda$ = 0.71073 Å), 2760 reflections, 2319 with F > 2$\sigma$ (F) used for refinement, R = 0.0422, wR = 0.1208.
14. Experimental data for [ReCl(pica)$(CO)_2$(NO)] Cl: IR ($CH_2Cl_2$): $\upsilon$ [$cm^{-1}$] = 2113, 2049 (vs, CO), 1795 (vs, NO); MS (FAB$^+$, DMF): 416 ($M^+$), 388, 307; $^1$H-NMR ($CDCl_3$): $\delta$ [ppm] = 8.83 (m, 1 H, H-6), 7.84 (m, 1 H, H-4), 7.54 (m, 1 H, H-3), 7.10 (m, 1 H, H-5), 4.83 (m, 1H, H-7), 4.73 (m, 2H, -$NH_2$), 4.46 (m, 1H, H-7)
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